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Metabolic Evidence for Stelar Anoxia in Maize Roots Exposed to Low O_2 Concentrations[1]

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ABSTRACT

This investigation presents metabolic evidence to show that in 4- to 5-day-old roots of maize (*Zea mays* hybrid GH 5010) exposed to low external O_2 concentrations, the stele receives inadequate O_2 for oxidative phosphorylation, while the cortex continues to respire even when the external solution is at zero O_2 and the roots rely solely on aerenchyma for O_2 transport. Oxygen uptake rates (micromoles per cubic centimeter per hour) declined at higher external O_2 concentrations in excised segments from whole roots than from the isolated cortex; critical O_2 pressures for respiration were greater than 0.26 moles per cubic meter O_2 (aerated solution) for the whole root and only 0.075 moles per cubic meter O_2 for the cortex. For plants with their shoots excised and the cut stem in air, ethanol concentrations (moles per cubic meter) in roots exposed to 0.06 moles per cubic meter O_2 were 3.3 times higher in the stele than in the cortex, whereas this ethanol gradient across the root was not evident in roots exposed to 0 moles per cubic meter O_2. Alanine concentrations (moles per cubic meter) in the stele of roots exposed to 0.13 and 0.09 moles per cubic meter O_2 increased by 26 and 44%, respectively, above the levels found for aerated roots, whereas alanine in the cortex was unchanged; the increase in stelar alanine concentration was not accompanied by changes in the concentration of free amino acids other than alanine. For plants with their shoots intact, alcohol dehydrogenase and pyruvate decarboxylase activities (micromoles per gram protein per minute) in roots exposed to 0.13 moles per cubic meter O_2 increased in the stele by 40 to 50% over the activity in aerated roots, whereas there was no appreciable increase in alcohol dehydrogenase and pyruvate decarboxylase activity in the cortex of these roots. More convincingly, for roots receiving O_2 solely from the shoots via the aerenchyma, pyruvate decarboxylase in the cortex was in an "inactive" state, whereas pyruvate decarboxylase in the stele was in an "active" state. These results suggest that for roots in O_2-free solutions, the aerenchyma provides adequate O_2 for respiration in the cortex but not in the stele, and this was supported by a change in pyruvate decarboxylase in the cortex to an active state when the O_2 supply to the roots via the aerenchyma was blocked.

The concept of O_2-deficient cores of tissue developing in roots exposed to low O_2 supply was suggested by Berry and Norris (4), based on studies on O_2 uptake of onion roots, and was later elaborated in the models of Armstrong and Beckett (2). Armstrong and Beckett (2) predicted that O_2 gradients across the root are gradual in the cortex due to this tissue's low O_2 demand and high porosity, and steep in the stele due to this tissue's high O_2 demand and low porosity. However, oxidative phosphorylation by cells in the root core would not be limited until O_2 inside the inner tissue dropped to very low levels (K_m of Cyt oxidase = 24×10^{-6} mol m^{-3} O_2; Yocum and Hackett [25]), when the metabolic state of cells in the core would change from aerobic[2] to anoxic. This sudden change in metabolic state of the tissue with respect to ATP synthesis would occur even if there were alternate oxidases present with a higher K_m for O_2 than Cyt oxidase.

No substantial metabolic evidence has been presented to establish the existence of an anoxic stele in roots. The key to the elucidation of its existence is the separate sampling of the cortex and the stele. Separation of the stele from the cortex has been used successfully for maize in studies on ion transport to the shoot (26) and radial differences in root respiratory O_2 demand (3), and for pea roots in studies on carbohydrate metabolism (23). In the latter case, it was demonstrated that the separation of the stele and cortex was remarkably complete.

In this study, we use indicators of anaerobic catabolism to show conclusively that at low O_2 supply, roots of maize (*Zea mays* hybrid GH 5010) have an aerobic cortex and an anoxic stele. Alanine is strongly accumulated in response to anaerobiosis (16, 19), and in the present experiment levels of alanine and ethanol were shown to be higher in the stele than in the cortex of roots exposed to a range of low external O_2 concentrations. Furthermore, in roots relying solely on O_2 transport via the aerenchyma, the key enzyme of alcoholic fermentation, PDC, was mostly in the "active" state in the stele and the "inactive" state in the cortex (*cf.* Morrell *et al.* [15]).

MATERIALS AND METHODS

Chemicals

Buffers, coupling enzymes, cofactors, and substrates were from Sigma Chemical Co. Sephadex was from Pharmacia (Uppsala, Sweden). All other reagents were analytical or reagent grade.

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[2] Abbreviations: aerobic cells, cells receiving sufficient O_2 for maximum rates of ATP synthesis via oxidative phosphorylation; anoxic cells, cells receiving no O_2 so ATP is synthesised entirely via substrate phosphorylation; PDC, pyruvate decarboxylase (EC 1.1.1.1); ADH, alcohol dehydrogenase (EC 4.1.1.1); TPP, thiamine pyrophosphate; COPR, critical O_2 pressure for respiration.

Growth of Plant Material

Seeds of maize (*Zea mays* hybrid GH 5010) were imbibed for 14 h in aerated deionized water and then germinated in the dark on plastic mesh suspended over aerated 0.5 mol m^{-3} $CaSO_4$ solution. Three days after germination, the seedlings were supplied with nutrient solution containing (mol m^{-3}): K^+ 0.6; Na^+ 0.6; NH_4^+ 0.3; Mg^{2+} 0.2; Ca^{2+} 0.5; NO_3^- 0.6; $H_2PO_4^-$ 0.3; Cl^- 1.6; SO_4^{2-} 0.2; pH was 5.2. Microelements were (mmol m^{-3}): B 8.9; Mn 1.9; Mo 0.023; Zn 0.15; Cu 0.06: The temperature throughout germination, growth, and treatments was 25°C.

Four days after imbibition, O_2 treatments were commenced and these treatments are described in detail in the figure and table legends. The appropriate O_2 concentrations in the nutrient solutions were imposed and maintained by bubbling with mixtures of air, O_2, and N_2 gas. Oxygen concentrations were measured using a Simplair F dissolved O_2 and temperature meter (John Morris Scientific Pty. Ltd), and in fully aerated solutions at 25°C were 0.26 mol m^{-3}.

Oxygen Uptake

Roots were separated into stele and cortex to within 30 mm of the root apex. A Clark O_2 electrode (Rank Bros., Cambridge, UK) was then used to measure O_2 uptake by the isolated cortex and whole root segments. Rates of O_2 uptake *versus* O_2 concentration were obtained using between 0.025 and 0.04 g fresh weight of tissue in 3.0 cm^3 of aerated nutrient solution at 25°C; the O_2 was depleted over 1 to 2 h.

Oxygen uptake was expressed on a protein basis. Proteins were assayed in the tissue after precipitation in 5% $HClO_4$ and then solubilizing the residue in 1.0 cm^3 of 2 N NaOH solution by incubating for 2 h at 70°C. A 0.15 cm^3 aliquot was used for the protein determination (13).

End Products of Anaerobic Catabolism

Approximately 0.3 g fresh weight of stele and cortex was ground for 1 min in 1.5 cm^3 of 5% $HClO_4$ and a pinch of acid-washed sand, and then centrifuged for 20 min at 30,000*g* and the supernatant withdrawn. Samples of root extract and treatment solution were neutralized with between 0.14 and 0.17 cm^3 69% (w/v) K_2CO_3 and centrifuged again for 20 min at 30,000*g*. Aliquots of supernatant were assayed for ethanol (5), alanine (22), and free amino acids (24).

Recovery was checked by adding known amounts of metabolites to the tissue. Recoveries from aerobic tissue were: for ethanol, 82% (whole root), 112% (cortex), and 111% (stele); for alanine, 81% (whole root), 106% (cortex), and 114% (stele); and for free amino acids, 83% (whole root), 106% (cortex), and 112% (stele). Solutions without roots, and containing ethanol at similar concentrations to those with plants, lost about 25% of their ethanol after 4 h of gas bubbling.

Metabolite concentrations in the root tissue were expressed on a water basis (mol m^{-3}) by using a water:protein ratio (w/w) of 83, 100, and 26 measured for the whole root, cortex, and stele, respectively. Water in the free space was deducted in making the above calculations and was assumed to be 15% for the whole root and cortex, and 5% for the stele (estimates of apparent free space are between 11 and 18% for nodal roots of wheat; Buwalda *et al.* [6]).

ADH and PDC

Tissue Extraction

Approximately 0.3 g fresh weight of stele and cortex was ground for 2 min at 3°C with 1.5 cm^3 of extraction buffer, 100 mg acid-washed Polyclar, and a pinch of acid-washed sand. The buffer was at pH 6.8 and contained (mol m^{-3}): Mes 125; Na^+ 47; Mg^{2+} 5; Cl^- 10; EDTA 2; DTT 2; TPP 0.5. Samples of crude extract were then centrifuged for 15 min at 30,000*g* and the supernatant withdrawn and assayed for ADH and PDC.

In other experiments, desalted root extracts were prepared by first grinding the root samples for 30 sec at 3°C in 1.5 cm^3 of extraction buffer at pH 7.4 and containing (mol m^{-3}): Tes 40; Na^+ 72; Mg^{2+} 5; Cl^- 60; EDTA 2; DTT 2; TPP 0.5. The extract was then centrifuged for 1 min at top speed in a Beckman 12 microfuge and the supernatant passed through a Sephadex G-25 column, 14 mm × 80 mm, using slight pressure. The column was equilibrated with buffer at pH 7.4 containing (mol m^{-3}): Tes 15; Na^+ 62; Mg^{2+} 3; Cl^- 56; EDTA 2; DTT 2; TPP 0.5. A 0.2 cm^3 aliquot of the desalted extract was immediately assayed for PDC at pH 6.0. Other aliquots of the extract were adjusted to pH 6.0 using one volume of 180 mol m^{-3} Mes, pH 6.0, to two volumes of desalted extract and assayed for PDC after 1 h incubation at 25°C.

Assay

ADH and PDC in the supernatant were assayed spectrophotometrically (340 nm) at 25°C. A 0.02 and 0.10 cm^3 aliquot was used for the ADH and PDC assays, respectively. The ADH assay mixture was at pH 7.0 and contained in a final volume of 1.0 cm^3 (mol m^{-3}): Tes 50; Na^+ 20; NADH 0.17; acetaldehyde 10. The PDC assay mixture was at pH 6.0 and contained in a final volume of 1.0 cm^3, 10 units yeast ADH and in mol m^{-3}: for crude extracts, Mes 50; Na^+ 30; Mg^{2+} 1; Cl^- 2; TPP 0.5; NADH 0.17; oxamate 50; pyruvate 10; for desalted extracts, Mes 80; Na^+ 37; Mg^{2+} 1; Cl^- 2; DTT 10; TPP 0.5; NADH 0.17; oxamate 50; pyruvate 10.

Recovery was checked by adding known amounts of purified enzyme to the tissue. Recoveries from aerobic samples were: for ADH, 80% (whole root), 75% (cortex), and 77% (stele); and for PDC, 110% (whole root), 115% (cortex), and 92% (stele).

Enzyme activities were expressed on a soluble protein basis. Soluble proteins in the supernatant were precipitated with an equal volume of 10% $HClO_4$ and then assayed by the Lowry method (described above).

RESULTS AND DISCUSSION

Oxygen Uptake by Isolated Cortex

Oxygen uptake by excised root segments, measured using Clark O_2 electrodes, was used to estimate COPRs. Average values were approximately 0.075 mol m^{-3} O_2 for the isolated cortex and >0.26 mol m^{-3} O_2 for the whole root (Fig. 1). The

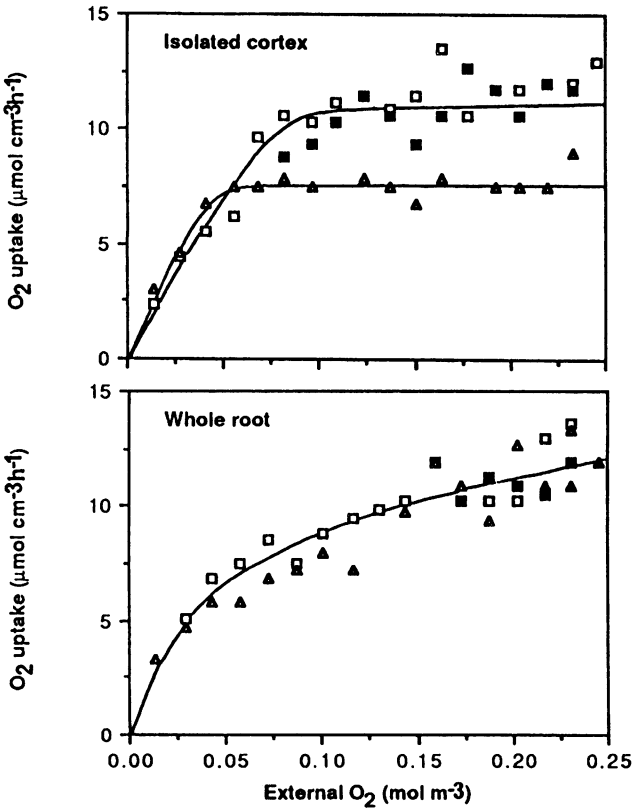

Figure 1. Oxygen uptake by segments of isolated cortex at a range of O_2 concentrations. Roots are from 4-d-old intact seedlings raised in aerated solution. Assay one (squares) and assay two (triangles) are replicates that were determined from 15 to 20 root segments. Closed symbols represent samples that were reaerated after 1 to 2 h.

higher COPR for the whole root segments than for the isolated cortex is likely to be due to O_2 diffusion limiting O_2 uptake by the inner cells of the root. However, these data cannot be used as convincing evidence for an inhibition of oxidative phosphorylation in the stele because the high COPR for the whole root may be due to the activity of alternate oxidases with a low affinity for O_2.

In subsequent experiments, it was decided on the basis of these COPRs to impose the O_2 treatments 0.09 and 0.06 mol m^{-3} O_2 to optimize the chance in intact roots that the cortex is aerobic, while most or all of the stele is anoxic. This range of O_2 concentrations was used because the O_2 uptake data on which it was based was determined for excised root segments submerged in solution. For intact roots, the COPR would be lower than was observed for excised roots because of the additional O_2 supply from the shoots via the aerenchyma. Also, the COPR of the submerged root cortices would be overestimated if there was a substantial flooding of the tissue and/or boundary layer resistance (see "Appendix" for detail).

End Products of Anaerobic Catabolism in Stele *versus* Cortex

Levels of end products of anaerobic catabolism were measured for roots that were pretreated for 12 h at 0.13 mol m^{-3} O_2. Pretreatment at low external O_2 concentrations ensures a high activity of ADH and PDC in maize root tissues (*cf.* Wignarajah and Greenway [21]). In this case, a 12 h pretreatment at 0.13 mol m^{-3} O_2 was imposed because this O_2 concentration is well above the COPR for the isolated cortex (see Fig. 1), yet maize roots have high activities of ADH and PDC even at 0.16 mol m^{-3} O_2 (21).

Levels were also measured for roots that were treated in nutrient solution containing 350 kPa sorbitol with their shoots excised and the cut stem in air. Sorbitol was added in order to reduce osmotically the water flow to the xylem, and for roots at low O_2 this treatment was found to increase both endogenous ethanol concentrations and leakage of ethanol to the external medium (Table I). In an early study, it was shown that there was no substantial effect on respiration after sudden immersion of maize root tissues in a solution with an osmotic pressure of 580 kPa (11).

Ethanol concentrations (mol m^{-3}) in these roots exposed to 0.06 mol^{-3} O_2 were 3.3 times higher in the stele than the cortex, while for roots exposed to 0 mol m^{-3} O_2 ethanol concentrations were high and similar in the stele, cortex, and external solution (Table II). There was no ethanol produced by roots exposed to 0.26 and 0.52 mol m^{-3} O_2 (Table II).

Alanine concentrations (mol m^{-3}) in the stele of roots exposed to 0.13 and 0.09 mol m^{-3} O_2 increased by 26 and 44%, respectively, above the levels found for aerated roots, whereas alanine in the cortex was unchanged (Table II). At 0 mol m^{-3} O_2, alanine concentrations in both the stele and cortex increased by 107 to 114% above the concentration in aerated roots (Table II). Alanine levels in aerated roots were only slightly higher than for roots exposed to 0.52 mol m^{-3} O_2 (Table II).

Net increases in alanine concentration in roots exposed to 0.06 mol m^{-3} O_2 were not accompanied by a proportional increase in the concentration of free amino acids, excluding alanine (Table III).

Table I. *Effect of Slowing of Waterflow in the Xylem on Ethanol Concentrations in the Stele and Cortex of Roots Exposed to Low O_2 Supply*

Roots of 5-d-old intact seedlings were raised for 4 d in aerated solution and then pretreated for 12 h at 0.13 mol m^{-3} O_2. After pretreatment, shoots were excised with the cut stem in air and the roots placed in 35 cm^3 vessels (12 plants per vessel) containing nutrient solution and 350 kPa sorbitol. Oxygen concentrations of the solution in the vessels was 0.06 mol m^{-3}. After 8 h in the vessels, roots were removed from solution and the stele and cortex separated using gloves. Values are means of three replicates with four to eight roots per replicate. LSDs are given in the table.

Treatment	Ethanol			
	Whole root	Stele	Cortex	External
	mol m^{-3}			
Shoots intact	0	0.06	0	0.01
Shoots excised and roots in 350 kPa sorbitol	0.11	0.39	0.12	0.11
LSD (P = 0.05)	0.04	0.13	0.03	0.06

Table II. *Ethanol and Alanine Concentrations in the Stele and Cortex of Roots Exposed to Low O_2 Supply*

Roots of 5-d-old intact seedlings were raised for 4 d in aerated solutions and then pretreated for 12 h at either 0.52, 0.26 (aerated), or 0.13 mol m^{-3} O_2. After pretreatment, shoots were excised with the cut stem in air and the roots placed in 35 cm^3 vessels (12 plants per vessel) containing nutrient solution and 350 kPa sorbitol. Oxygen concentrations of the solutions in the vessels were the same as in the pretreatment, except for those roots pretreated at 0.13 mol m^{-3} O_2, which were transferred to solutions at either 0.13, 0.09, 0.06, or 0 mol m^{-3} O_2. After 8 h in the vessels, roots were removed from solution and the stele and cortex were separated using gloves. Values are means of three replicates with four to eight roots per replicate. LSDs are given in the table.

O_2		Ethanol				Alanine		
12 h of pretreatment	8 h in the vessels	Whole root	Stele	Cortex	External	Whole root	Stele	Cortex
		mol m^{-3}						
0.52	0.52	0	0.06	0	0	5.5	11.7	6.8
0.26 (aerated)	0.26	0	0	0.02	0	7.4	13.7	7.6
0.13	0.13	0.01	0	0.02	0	6.0	17.2	8.0
0.13	0.09	0	0.06	0.01	0	8.9	19.7	7.8
0.13	0.06[a]	0.11	0.39	0.12	0.11	—	—	—
0.13	0	0.36	0.34	0.43	0.42	12.3	29.3	15.7
LSD (P = 0.05)		0.06	0.06	0.03	0.06	1.2	1.5	1.0

[a] Results from a second experiment (see Table I).

ADH and PDC in Stele *versus* Cortex

ADH and PDC activities (μmol g $protein^{-1}$ min^{-1}) were measured for roots with their shoots intact. For roots exposed to 0.13 mol m^{-3} O_2, ADH and PDC activity in the stele increased by 40 to 50% over the activity in aerated roots, whereas there was no appreciable increase in ADH and PDC activity in the cortex of these roots (Table IV).

Characteristics of the key enzyme of ethanol synthesis, PDC, extracted from roots that were isolated and desalted at pH 7.4 and then transferred to pH 6.0 (as described by Morrell *et al.* [15]) are shown in Table V. In aerated roots, the PDC extracted from both the cortex and the stele was in an inactive state, *i.e.* the PDC had a long lag before maximum activity was reached during immediate assay at pH 6.0 and a large percentage increase in activity after 1 h incubation at pH 6.0. For roots that were in O_2-free solutions but with their shoots in air, the PDC in the cortex was in an inactive state, whereas the PDC in the stele was in an active state: the lag during immediate assay at pH 6.0 was 1.4 min in the cortex compared with 0.2 min in the stele, while the increase in PDC activity after 1 h incubation at pH 6.0 was 50% for the cortex and only 25% for the stele (Table V). Interestingly, when the roots and shoots of seedlings were both submerged in the O_2-free solution, the observed lag and percentage increase in PDC activity in the cortex was halved (Table V). The change in the PDC state of the cortex from inactive to active once the shoots were submerged is no doubt due to blocking of the O_2 transport that would occur when the shoots were in air.

Reversibility of the PDC state from inactive to active was also demonstrated by a significant reduction in the lag for PDC activity after 1 h incubation at pH 6.0. This reduction occurred for PDC extracted from roots exposed to 0.26 and 0.52 mol m^{-3} O_2, but not for roots exposed to 0 mol m^{-3} O_2 whose shoots were submerged in the O_2-free solution (Table VI). Clearly, the PDC is in an inactive state in all the tissues of the aerated roots. Even the small percentage activation of PDC in the stele of aerated roots and roots at higher O_2 was larger than for roots exposed to 0 mol m^{-3} O_2 (Table VI). This much smaller percentage activation in the extracts from the stele of roots exposed to 0.26 and 0.52 mol m^{-3} O_2 (Table VI), as compared with roots in the experiment presented in Table V, was presumably due to an artifact associated with the low level of PDC in the experiment presented in Table VI. At such low activities of PDC, it requires a long time to get an accurate estimate of the rate of the reaction. Hence, there would already be activation during the "immediate" assay, which would result in a small percentage activation after the 1 h incubation at pH 6.0.

GENERAL DISCUSSION

The possible existence of an anoxic stele in roots is clearly a central question in the elucidation of adaptation of plants to O_2 deficiency. This is particularly important because of the function of the stele in long distance transport of mineral nutrients to the shoots and of sugars to the roots. For example, anoxia of entire maize roots completely inhibited unloading of 2-deoxy-D-glucose from the phloem in the root tip (17).

Occurrence of Anoxic Stele and Aerobic Cortex

Our present results show conclusively that maize roots at low O_2 supply develop an anoxic stele and aerobic cortex. Such differences between tissues reflects the combination of the slower O_2 diffusion into the compact stelar tissues (10), the higher respiratory demand per unit volume of the stele compared with the cortex (23), and the greater distance of the stele from the O_2 source (3). Oxygen is readily supplied to the root cortex from the external solution and from the aerial

Table III. *Alanine and Free Amino Acid (Excluding Alanine) Concentrations in the Stele and Cortex of Roots Exposed to Low O_2 Supply*

Roots of 5-d-old intact seedlings were raised for 4 d in aerated solutions and then pretreated for 12 h at either 0.26 (aerated) or 0.13 mol m^{-3} O_2. After pretreatment, shoots were excised with the cut stem in air and the roots placed in 35 cm^3 vessels (12 plants per vessel) containing nutrient solution and 350 kPa sorbitol. Oxygen concentrations in the vessels were the same as in the pretreatment, except for those roots pretreated at 0.13 mol m^{-3} O_2, which were transferred to solutions at either 0.06 or 0 mol m^{-3} O_2. After 8 h in the vessels, roots were removed from solution and the stele and cortex were separated using gloves. Values are means of three replicates with four to eight roots per replicate. LSDs are given in the table.

O_2		Alanine			Free Amino Acids (Excluding Alanine)		
12 h of pretreatment	8 h in the vessels	Whole root	Stele	Cortex	Whole root	Stele	Cortex
		mol m^{-3}					
0.26 (aerated)	0.26	5.0	8.5	5.7	14.1	17.9	13.5
0.13	0.06	8.8	14.3	9.8	13.4	13.6	14.3
0.13	0	11.2	17.2	13.8	18.3	15.0	14.2
LSD (P = 0.05)		1.2	1.8	1.9	5.6	4.4	5.6

tissues via the aerenchyma. This latter possibility was demonstrated by a substantial delivery of O_2 from the shoots to the root tip of these maize plants, evaluated using a cylindrical platinum O_2 electrode, as described by Armstrong (1) (data not shown).

Metabolic evidence that shows the existence of an anoxic stele and aerobic cortex includes:

The PDC extracted from roots exposed to O_2-free solutions but with their shoots in air, which was in an inactive state in the cortex and an active state in the stele. This provides persuasive evidence for the hypothesis of Armstrong and Beckett (2) that the aerenchyma can supply adequate O_2 for respiration in the cortex while the stele remains anoxic.

The increase in ADH and PDC activity in the stele but not the cortex of roots exposed to 0.13 mol m^{-3} O_2, compared with aerated roots.

The increase in alanine concentration in the stele but not the cortex of roots exposed to 0.13 and 0.09 mol m^{-3} O_2, compared with aerated roots. This increase in alanine concentration was independent of changes in the concentration of free amino acids, excluding alanine, so presumably alanine accumulated as part of a shift from aerobic to anaerobic catabolism and not as a consequence of decreased protein synthesis.

The higher ethanol concentration in the stele than in the cortex of roots exposed to 0.06 mol m^{-3} O_2, whereas this ethanol gradient across the root did not occur in roots exposed to O_2-free solutions.

Possible Ethanol Consumption by Aerobic Cortex

One can speculate on the possible mechanisms of ethanol disposal by the anoxic stele. Clearly, ethanol is leaked from the root to the external medium even though at the end of the experiment ethanol concentrations in the cortex and bathing solution were similar (*cf.* Tables I and II). Presumably, the cortical cells facing the free space of the roots would only require a small concentration gradient for leakage in the case of a compound like ethanol, for which the membranes have a high permeability (reviewed by Davies [8]).

Ethanol is also removed in the stele via the xylem stream, as was shown by an accumulation of ethanol in the roots of plants whose waterflow to the xylem was slowed (*cf.* Table I).

Table IV. *ADH and PDC Activities in the Stele and Cortex of Roots Exposed to Low O_2 Supply*

Roots of 5-d-old intact seedlings were raised for 4 d in aerated solution and then exposed for a further 12 h to either 0.26 (aerated) or 0.13 mol m^{-3} O_2. Values are means of three replicates with four to eight roots per replicate. LSDs are given in the table.

O_2	Enzyme Activity					
	ADH			PDC		
	Whole root	Stele	Cortex	Whole root	Stele	Cortex
mol m^{-3}	*μmol g protein^{-1} min^{-1}*					
0.26 (aerated)	839	887	831	100	65	123
0.13	1305	1323	916	130	91	122
LSD (P = 0.05)	221	242	109	20	13	22

Table V. *Characteristics of PDC at pH 6.0 Extracted from the Stele and Cortex of Roots that Were Pretreated at Low O_2 Concentrations*

Roots of 5-d-old intact seedlings were raised for 4 d in aerated solution and then pretreated for 12 h at 0.13 mol m^{-3} O_2. After pretreatment, roots were exposed for a further 6 to 8 h to nutrient solution at either 0.26 (aerated) or 0 mol m^{-3} O_2. Roots at zero O_2 either had their shoots in air or their shoots submerged in the O_2-free solution. Values are means ± SE of two replicates with four to eight roots per replicate.

Characteristic	Roots at 0.26 mol m^{-3} O_2 with Shoots in Air		Roots at 0 mol m^{-3} O_2 with Shoots in Air			Roots at 0 mol m^{-3} O_2 with Shoots in O_2-free Solution		
	Stele	Cortex	Whole root	Stele	Cortex	Whole root	Stele	Cortex
Lag (min) during immediate assay	1.1 ± 0.1	1.9 ± 0.2	1.8 ± 0	0.2 ± 0.2	1.4 ± 0.1	0.2 ± 0.2	0.3 ± 0.3	0.7 ± 0.6
Maximum activity (OD min^{-1})								
Immediate assay	0.016	0.028	0.036	0.012	0.030	0.069	0.020	0.056
After 1 h incubation	0.026	0.047	0.052	0.015	0.045	0.075	0.024	0.069
Percentage increase in activity after 1 h incubation	63	68	44	25	50	9	20	23

A rough estimate of the extent to which this may occur is as follows: maize transpires at a rate of 400 cm^3 g^{-1} dry weight produced (estimated for C_4 plants by Ludlow [14]) and has a relative growth rate of 0.2 d^{-1} (dry weight basis). Assuming a root:shoot ratio of 0.5, the daily waterflow through the root during a light period of 12 h would be 240 cm^3 g^{-1} dry weight of root or 720 cm^3 g^{-1} dry weight of stele (stele dry weight is estimated to be 33% of root dry weight). With the further assumption that dry weight is 10% of fresh weight, one can calculate the rate of waterflux to be 72 cm^3 g^{-1} fresh weight of stele 12 h^{-1}, so the volume of water containing ethanol in the stele would be replaced every 10 min.

Close proximity of an aerobic cortex and an anoxic stele raises the possibility of ethanol that was produced by the anoxic stele being oxidized by the aerobic cells of the cortex. This was assessed by assuming that ethanol production in the stele and cortex of roots at zero O_2 is similar on a protein basis. For the 12 roots exposed for 8 h to O_2-free solutions, net ethanol production was 14.8 μmol by the whole root and 4.9 μmol by the stele (calculated using data from Table II; stele protein weight is estimated to be 33% of the root protein weight). In comparison, the same number of roots exposed for 8 h to 0.06 mol m^{-3} O_2 had a net ethanol production of 3.9 μmol. Assuming that in the roots exposed to 0.06 mol m^{-3} O_2 only the stele was anoxic and produced ethanol at equal rates to the stele of roots in O_2-free solutions, then the 1.0 μmol shortfall in the amount of measured ethanol produced by these roots might be due to ethanol being consumed by aerobic cells of the cortex. Therefore, in the present roots where xylem flow was stopped osmotically by sorbitol in the external solution, ethanol oxidation in the cortex would prevent 20% of carbon loss which would otherwise occur due to ethanol leakage from the roots to the medium. The consequences of this flow of ethanol back to the respiratory and synthetic pathways to the carbon and energy balance of the plant, as well as the possible exchange of other metabolites between the anoxic stele and the aerobic cortex, is currently being investigated in this laboratory.

Table VI. *Characteristics of PDC at pH 6.0 Extracted from the Stele and Cortex of Roots that Were Pretreated in Aerated Solutions*

Roots of 5-d-old intact seedlings were raised in aerated solution and then exposed for a further 6 to 8 h to nutrient solution at either 0.52, 0.26 (aerated), or 0 mol m^{-3} O_2. Roots at zero O_2 had their shoots submerged in the O_2-free solution. Values are means of two replicates with four to eight roots per replicate. LSDs are given in the table.

Characteristic	Roots at 0.52 mol m^{-3} O_2 with Shoots in Air			Roots at 0.26 mol m^{-3} O_2 with Shoots in Air			Roots at 0 mol m^{-3} O_2 with Shoots in O_2-free Solution		
	Whole root	Stele	Cortex	Whole root	Stele	Cortex	Whole root	Stele	Cortex
Lag (min)									
Immediate assay	2.5	1.7	1.9	2.8	1.4	1.7	0.7	0.1	0.3
After 1 h incubation	0.7	0.8	0.4	1.4	0.6	0.8	0.1	0.1	0.3
LSD (P = 0.05)	1.5	0.2	0.6	1.2	0.8	0.2	0.8	0.3	0.6
Maximum activity (OD min^{-1})									
Immediate assay	0.008	0.0035	0.007	0.007	0.0036	0.009	0.020	0.007	0.018
After 1 h incubation	0.012	0.0038	0.010	0.013	0.0040	0.012	0.020	0.007	0.018
Percentage increase in activity after 1 h incubation	50	9	43	86	11	33	0	0	0

Relevance to Crafts-Broyer Theory

The Crafts-Broyer hypothesis is not a central issue of this investigation, yet some comment needs to be made on the possible relevance of our data obtained in fully aerated solutions to this theory. Crafts and Broyer (7) proposed that ion transport across the root culminates in a passive leakage of ions into the xylem, because even in aerated solutions the interior of the root receives inadequate O_2 for oxidative phosphorylation, causing the stelar parenchyma cells to lose ions.

The present results for maize roots give no clear answer to this question. Ethanol accumulation and increased ADH and PDC activities in the stele indicate a switch from aerobic to anaerobic pathways at much lower external O_2 concentrations than in aerated solutions. Yet, a COPR for the whole root at O_2 concentrations greater than in air, together with a significant alanine accumulation in the stele of roots exposed to 0.26 compared with 0.52 mol m^{-3} O_2, suggests that the stele is always under a certain degree of O_2 deficiency.

CONCLUSION

Metabolic evidence has been presented that supports the hypothesis that at low O_2 supply, maize roots develop an anoxic stele and an aerobic cortex. In our experiments, the low O_2 condition most relevant to plant growth and function in waterlogged soils was when the roots were made solely dependent on O_2 supply via the aerenchyma. In this treatment, the state of the key enzyme of alcoholic fermentation, PDC, strongly indicates there was adequate O_2 for oxidative phosphorylation in the cortex while the stele remained anoxic.

Our current findings raise the possibility of stelar anoxia in subapical regions of the root coexisting with an apical region still maintained in an aerobic state by O_2 transport from the shoot. How this combination affects stelar function is not yet well understood. In previous studies, it was shown that roots relying solely on O_2 supplied via the aerenchyma retained high adenylate energy charge and ATP/ADP ratios (for maize, Drew *et al.* [9]) and continued to elongate (for rice, pea, and pumpkin, Webb and Armstrong [20]; for wheat, Thomson *et al.* [18]). However, the effects of anoxia in the stele on its function is best gauged by data on ion transport. For example, net K^+ transport to the shoots in aerenchymatous nodal roots of wheat exposed to 0.003 mol m^{-3} O_2 in nutrient solution was inhibited by 28 to 40%, as assessed from a comparison with rates of uptake by roots transferred from low O_2 to air (P. Kuiper, personal communication); the latter rates would give a good estimate of the capacity of the roots to transport ions. Similar inhibitions ranging between 40 and 60% were found for transport of radioactively labeled K, P, and Cl to the shoots of rice grown in O_2-free nutrient solutions (12). Such data indicate that roots with an anoxic stele allow ion transport to the shoots, albeit at rates well below the maximum capacity of the transport systems. Rigorous proof for this hypothesis is required by measurement of ion transport and the metabolic state of the stele in the same roots.

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APPENDIX

Predicted Effect of Flooding of Root Tissue and Boundary Layer Resistance on the COPR of Submerged Cortical Segments

The multicylindrical models of Armstrong *et al.* (3) can be used to calculate the effect of flooding in the inner/middle cortex on the potential O_2 deficit across the submerged cortical segments. By assuming that the water filling the hollow centers of the cortical segments remains unstirred by the agitation within the respirometer, the predicted O_2 deficit across the root cortex (Δc) is calculated to be 0.040 mol m^{-3} O_2 from the equation (modified from Equation 8 of Armstrong *et al.* [3]):

$$\Delta c = \frac{Qc}{4\,Dc}\, rs^2 \left\{\frac{rc^2}{rs^2} + 2\, log_e\left(\frac{rs}{rc}\right) - 1\right\}$$

where Dc = O_2 diffusivity of cortex of 8.6×10^{-2} cm^2 h^{-1}; Qc = cortical respiration of 9.3 μmol cm^{-3} h^{-1} (*cf.* Fig. 1); rc = cortical radius of 0.054 cm; rs = stelar radius of 0.023 cm.

If there is a boundary layer of at least 0.008 cm at the cortical segment surface despite the stirring in the O_2 electrode, then a further O_2 deficit across the boundary layer (Δb) of 0.020 mol m^{-3} O_2 can be calculated from the equation (modified from Equation 10 of Armstrong *et al.* [3]):

$$\Delta b = \frac{1}{2\,Db}\left\{Qc\,(rc^2 - rs^2)\right\} log_e\left(\frac{rb}{rc}\right)$$

where Db = O_2 diffusivity of boundary layer of 7.6×10^{-2} cm^2 h^{-1} and rb = boundary layer radius of 0.062 cm.

The total of these combined O_2 deficits of 0.060 mol m^{-3} O_2 is reasonably close to our experimental finding of 0.075 mol m^{-3} O_2 for the COPR of the isolated cortex.

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